Abstract

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Project Title: Pharmacological inhibitors of tissue-nonspecific alkaline phosphatase (TNAP)

Abstract: DESCRIPTION (provided by applicant): The mechanisms that regulate tissue calcification are of major importance, as they ensure that calcification of the skeleton proceeds normally while mineralization is prevented elsewhere in the body. Alterations in these regulatory mechanisms, either due to genetic defects or as a result of aging, lead to disease, such as osteoarthritis and arterial calcification. Inorganic pyrophosphate (PPi) is a potent inhibitor of calcification and three molecules have been identified as central regulators of mineralization via their ability to control the pool of extracellular PPi, i.e., PPi is generated outside of the cells by the enzymatic action of nucleotide pyrophosphatase phosphodiesterase 1 (NPP1) and it is also transported from the inside to the outside of the cells by the ankylosis protein (ANK). In turn, extracellular PPi is degraded by the enzymatic action of tissue-nonspecific alkaline phosphatase (TNAP). Genetic experiments in mice have revealed that the functional deletion of the NPP1 or the ANK gene lead to very similar disease states that include osteoarthritis, fusion of the ligaments of the spine and arterial calcification. The simultaneous inactivation of the TNAP gene in NPP1-deficient and ANKdeficient mice leads to normalization of extracellular PPi levels, and correction of calcification abnormalities in these mice. The central hypothesis underlying this research proposal is that the pharmacological ablation of TNAP function will lead to an increase in the concentrations of extracellular PPi that will result in amelioration/prevention of arterial calcification. Since arterial calcification is a condition associated with the development of atherosclerosis, we will test this hypothesis using the NPP1-deficient model of osteoarthritis and arterial calcification described above, but also the Apolipoprotein E-deficient mouse model that mimics the development of atherosclerotic plaques as seen in humans. As a first step toward targeting TNAP therapeutically, we have optimized a microtiter plate enzymatic assay using pnitrophenylphosphate as substrate and measuring liberated p-nitrophenol as product, confirming suitable assay performance for the high-throughput environment. We have screened libraries containing 53,080 compounds and succeeded in identifying small molecule, drug-like lead compounds that can be further modified to obtain highly specific TNAP inhibitors for in vivo use. Initial hits from this screen were counter-screened using very similar assay condition but using PPi as substrate and using the Biomol reagent to detect liberated phosphate as product. Subsequent tests will also be run using both assay designs to select those compounds that inhibit TNAP but not other related human phosphatases or NPP1. We propose to use both of these already optimized assays for screening the NIH's chemical library. We will use a combination of computer modeling, docking experiments and chemical synthesis to further modify the HTS hits to design novel, improved inhibitors of TNAP activity for use as in vivo therapeutics and to test these novel TNAP inhibitors in vivo for their ability to ameliorate/prevent arterial calcification in our animal models of arterial calcification and atherosclerosis.

Thesaurus Terms:
High throughput screening, tissue-nonspecific alkaline phosphatase, TNAP, tissue calcification, osteoarthritis, arterial calcification, Inorganic pyrophosphate, PPi, nucleotide pyrophosphatase, phosphodiesterase 1, NPP1, ankylosis protein, ANK, Apolipoprotein E-deficient mouse model, enzymatic

assay, pnitrophenylphosphate, p-nitrophenol, Biomol reagent, assay, computer modeling, docking experiments, chemical synthesis, atherosclerosis

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